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Antibacterial Activity of Pseudomonas Aeruginosa ISP1RL3 Against Multidrug Resistance Bacteria

Anak Agung Gede Indraningrat[1*], Pande Putu Christine Putri Purnami[1], Anak Agung Sri Agung Aryastuti[2], Made Dharmesti Wijaya[2]

[1] Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar, Indonesia.
[2] Department of Pharmacology, Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar, Indonesia.

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Corresponding Author:
Anak Agung Gede Indraningrat
indraningrat@warmadewa.ac.id

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Abstract: Seaweed-associated bacteria have a pivotal role to synthesize arrays of secondary metabolites. This study described a bacterial isolate encoded as ISP1RL3 that was isolated from seaweed Eucheuma cottonii. Ethyl acetate extracts of ISP1RL3 was screened against non-multidrug bacteria (Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603) and multi-drug resistance bacteria (Methicilin-resistance S. aureus (MRSA), E. coli ESBL, K. pneumoniae ESBL, dan A.baumanii ESBL). Our result showed that ISP1RL3 displayed rod structure, Gram negative and identified as Pseudomonas aeruginosa. The crude extract displayed strong antibacterial activity against all bacterial test with the range zone of inhibition of 10 mm – 18 mm. The GC-MS analysis detected the presence of 13 antibacterial compounds with four dominant moleculer were o-Xylene, Ethylbenzene, p-Xylene and Benzene, 1,3-dimethyl. Overall, this finding highlights the potency of seaweed-associated bacteria to synthesize active compounds against multidrug resistance bacteria.

Keywords: Antibacterial Activity; ISP1RL3; Pseudomonas Aeruginosa; Resistance Bacteria

Introduction

The incidence rate of multidrug-resistance (MDR) bacteria is increasing significantly in recent year which pose a very serious threat to human health (Bharadwaj et al., 2022). A number of bacteria develop resistance due to overuse and misuse of antibiotics in society which trigger accumulation of multiple genes in bacterial plasmids (Urban-Chmiel et al., 2022). β-lactamase-resistant Streptococcus pneumoniae, methicillin-resistant Staphylococcus aureus (MRSA), and vancomycin-resistant Enterococcus faecium (VRE) are examples of Gram-positive bacteria associated with multi-drug resistance (Jubeh et al., 2020). On the other hand, the broad spectrum beta-lactamase (ESBL) enzymes found in Enterobacteriaceae also exacerbate the threat of antibiotic resistance globally (Amankwah et al., 2022). Despite the importance to educate society to consume antibiotics wisely, explorations to find a stronger antibiotic producers remain crucials to combat MDR bacteria.

The search for (novel) antibiotics producers have for decades been focused on terrestrial organisms especially microorganisms, mainly from actinobacterial and fungal group (Schneider, 2021). However, a number of studies showed the hight of de-replication whereas the same active compounds were re-discovered from terrestrial bacteria, thus it reduces the novelty rate of compounds (De La Hoz-Romo et al., 2022). Marine habitats, on the other hand, provide diverse sources of bioactive molecules including antibiotics which are of pharmaceutically important (Hai et al., 2021). A number of marine organisms such as sponges, corals, nudibranch and seaweeds have been reported to synthesize pharmaceutical important compounds including antibiotics (Tan, 2023). Clinical testing and

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optimization of biosynthesis of marine-derived active compounds are often limited by huge biomass that is required to obtain an ample amount of extract (Negara et al., 2016). Whereas, direct cultivation of large biomass from nature is not feasible due to ecological issues. Therefore, bioprospecting of marine bioactive molecules has now been focused on marine bacteria especially that are associated with marine organisms (Srinivasan et al., 2021). Bacteria offer fast and relatively easy cultivability under laboratorium conditions which could be directed to synthezise a compound of interest (Bengtsson-Palme, 2020).

Eucheuma cottonii is one of the seaweed species that is commonly cultivated in Indonesia because of its high nutrient contents and beneficial active compounds for food and pharmaceutical industy such as carragenan, flavonoids, triterpenoids, steroids, alkaloids and tannins (Andriani et al., 2016; Putri et al., 2019). Like many other seaweed species, E. cottonii build an intimate mutualistic relationship with many different bacteria. Seaweed-associated bacteria contribute in growth, morphogenesis, and protection by synthesizing various bioactive molecules including antibacterial compounds (Singh & Reddy, 2014). Unlike its host, studies on E. cottonii-associated bacteria are still limited (Hafsan et al., 2019; Purnami et al., 2022). A previous study reported isolation of Aeromonas sp. that inhibit S. aureus and E. coli. Furthemore, a recent study has reported 23 bacterial isolates from E. cottonii collected at the coastal waters of Buleleng, Bali with six of these isolate displayed antibacterial activity against S. aureus, S. mutans, E. coli, and K. pneumoniae (Purnami et al., 2022).

Among these six bacterial isolates that was previously described (Purnami et al., 2022), an isolate encoded as ISP1RL3 is one of the isolate with a strong antibacterial activity. However, up to know only a limited information is available on the bacterial isolate with regards to its species. Importantly, the antibacterial activity of the isolate need to be verified by employing chemical extraction and be tested against non resistance and MDR bacterial target . Thus, this study was designed to unravel antibiotic potential of the isolate ISP1RL3 against a panel of non resistance and MDR bacteria. Furthemore, morphological observation, molecular identification, phylogenetic tree analysis and chemical profiling via GC-MS were described.

Method

An intensive experimental works were performed at the Research Laboratory Faculty of Medicine and Health Sciences Warmadewa University from May to July 2023 which are described as follows:

Morphological characterization 16S rRNA gene sequencing and molecular identification

Genomic DNA of the isolate ISP1RL3 was isolated by emplying the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). Molecular identifification of the isolate was performed by was initiated by polymerase chain reactions by targeting 16S rRNA gene in a 50 µL reactions consisted of 25 µL My Taq HS Red mix 2x, 1 µL primer (20µM) 27F (5′-AGAGTTTGATCMTGGCTCAG-3′), 1 µL primer (20µM) 1492R (5′- CGGTTACCTTGTTACGACTT-3′), 22 µL DNA free water, and 1 µL DNA template. The PCR program consisted of pre-denaturation (95 °C for 2 minutes), 35 cycles of denaturation (95 °C for 1 minute), annealing (59 °C for 1 minute), extension (60 °C for 2 minutes), and final extension (72 °C for 2 minutes). The PCR product was run using 1% gel electrophoresis and the purified PCR product was sequenced bi-directionally at Genetika Science. The obtained sequence product was analyzed using the nucleotide BLAST (Basic Local Alignment Search Tool). Ten key base sequence data results from the BLAST search were recorded and compared. The phylogenetic tree was formed based on basic information for each isolate using the MEGA XI software (https://www.megasoftware.net) (Girão et al., 2019).

Morphological observation

Morphological features of the isolate ISP1RL3 was evaluated by performing Gram staining, followed by observation under light microscope (Leica DM750) at 1000 times magnification. To obtain a more comprehensive morphological data, a sample of ISP1RL3 isolate was observed under scanning electron microscope (SEM) which was performed at the Mero Foundation.

Bacterial fermentation and extraction

The biomass of ISP1RL3 was obtained by growing the culture in 100 mL liquid ISP-2 media for 14 days and shaken at 150 rpm. Cell mass and supernatant were separated using nitrocellulose membrane (Whatman paper no 1) and the filtrate was subsequently extracted twice with ethyl acetate(1:1, v/v). The solvent was separated with a separatory funnel and was evaporated with vacuum evaporator at 40oC to obtain ethyl acetate crude extract. The crude extract was weighed and dissolved in 1 mL of ethyl acetate (Sulistyani & Akbar, 2014).

Antibacterial screening

The crude ethyl acetate extract was then tested for antibacterial activity against nonresistant pathogenic and multidrug-resistant (MDR) bacteria. Four nonresistant pathogenic bacteria used as test bacteria were Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922; and Klebsiella pneumoniae ATCC 700603. Four MDR bacteria used as test bacteria were Methicilin-resistant Staphylococcus aureus

(MRSA), Escherichia coli and Klebsiella pneumoniae ESBL (Extended spectrum β-lactamase), and Acinetobacter. Screening for the antibacterial activity of the ethyl acetate extract isolate ISP1RL3 was carried out using the disc diffusion method. For each paper disc (6 mm in diameter), 20 μL of the ethyl acetate extract isolate ISP1RL3 was dropped onto a triplicate of sterile blank paper discs (6 mm in diameter) that had been placed on Luria-Bertani (LB) agar in a Petri dish that had been spread with 200 μL of liquid culture of multidrug-resistant bacteria. Petri dishes are incubated for 24 to 48 hours at 37°C. The inhibition zone is shown as the transparent area or clear zone that appears around the paper disc. The diameter of the clear zone formed was measured using digital caplipers recorded. Ethyl acetate was used as a negative control. Antibiotic levofloxacin 5 μg was used as a positive control. Diameter zone of inhibition was measured using a digital caliper with three replications.

Thin Layer Chomatography and GC-MS analysis

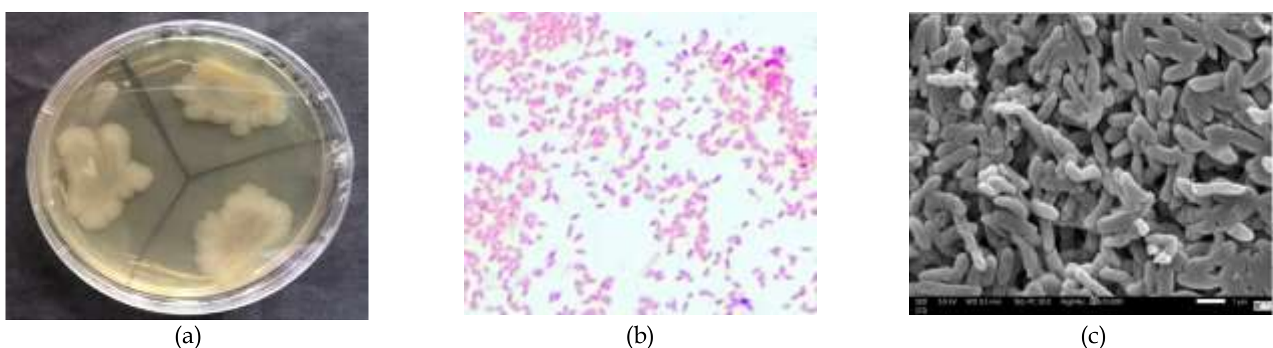
The separation and purification of the crude extract were analyzed by thin layer chromatography (TLC) by spotting the extract on a silica gel GF254 TLC plate with capillary tube and then eluting the TLC plate with n-hexane : ethyl acetate (4:6 v/v). The silica gel plate then observed under UV light 254 nm, and determine the Rf value of each fraction. After being visualized with UV 254 nm, each spot that appeared was scraped off the silica gel plate and dissolved with the eluent used, n-hexane: ethyl acetate (4:6) and then retested for antibacterial activity against non-resistant and MDR bacteria to confirm the obtained antibacterial activity in the first screening using the disc diffusion method. Brifely, blank sterile paper discs (6.0 mm in diameter) were dripped with 20 μL per fraction and then placed on LB media in a Petri dish that had been inoculated with the test bacteria. Petri dishes are incubated for 48 hours at 37°C.

Identification of the active compound from ISP1RL3 crude ethyl acetate extract was carried out using the gas chromatography-mass spectrometry (GC-MS) method. ISP1RL3 crude ethyl acetate extract of 0.1 gram was analyzed by GC-MS which was carried out at the Bali Police Forensic Laboratory. The chromatograms obtained from the GC-MS results were then analyzed by matching the compound fragments from each chromatogram peak with related literatures to determine the type of content and function of the detected bioactive compounds.

Result and Discussion

Result

ISP1RL3 bacterial isolate has a cell morphology with bacilli-shaped cells and is a type of Gram-negative bacteria which is indicated by red-stained cells under light microscopy (Figure 1a). ISP1RL3 bacterial isolate has slow growth, and catalase positive. The cell morphology of the ISP1RL3 bacterial isolate was observed very clearly under scanning electron microscopy (SEM) with bacillus-shaped cells, without spores or spore chains, with a smooth surface, and attached to each other among the surrounding cells (Figure 1b). Pure ISP1RL3 bacterial isolate aged 11 days grown on ISP-2 media has colonies with irregular colony shapes, firmly attached to the media, has colonial pigmentation with a grayish-white color accompanied by a rough and opaque colony surface as shown in Figure 1c.



(a) (b) (c)

Figure 1. Macroscopic and microscopic shapes of ISP1RL3 bacterial isolate: (a) Pure colonies of ISP1RL3 bacterial isolates aged 11 days on ISP-2 agar media; (b) Bacilli-shaped cells and stained red from the results of Gram staining with 1000x magnification ISP1RL3; (c). Bacterial cells based on SEM observations with 10000x magnification

DNA isolation of ISP1LR3 28.6 ng/μL. The DNA purity level of the ISP1RL3 bacterial isolate is 1.82 at the A260/280 nm ratio. The DNA band fragment of ISP1RL3 isolate is parallel to the DNA marker fragment at a size of around 1500 bp, which is 1400 bp. This indicates that the amplification site on the ISP1RL3 DNA corresponds to the 16S rRNA gene fragment that is the amplification target. ISP1RL3 bacterial isolate has a DNA sequence with a sequence homology level of 99.93% with the DNA sequence of the bacterium Pseudomonas aeruginosa strain AB18 (Table 2).

The top ten nucleotide BLAST showed that the isolate ISP1RL3 refers to *Pseudomonas aeruginosa* with the top hit assigned the isolate as *P. aeruginosa* strain AB18 (Table 1). Furthemore, phylogenetic tree confirmed this result (Figure 2).

Table 1. The BLASTn results of SMPRL-2 16S rRNA gene based on NCBI database

Description	Accession number	Query cover %	Percentage identity %	e-value	Max score
Pseudomonas aeruginosa strain AB18 16S ribosomal RNA gene, partial sequence	MT598026.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain M4 16S ribosomal RNA gene, partial sequence	MT180543.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain BD0603 16S ribosomal RNA gene, partial sequence	MT109313.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain PA0504 16S ribosomal RNA gene, partial sequence	MK607451.1	100	99.93	0.0	2580
Pseudomonas sp strain 16S_DB45 16S ribosomal RNA gene, partial sequence	MN889026.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain 16S_DB6 16S ribosomal RNA gene, partial sequence	MN889009.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain 16S_DB3 16S ribosomal RNA gene, partial sequence	MN889008.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain 16S_DB1 16S ribosomal RNA gene, partial sequence	MN889006.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain JY-11 ribosomal RNA gene, partial sequence	MK825339.1	100	99.93	0.0	2580
Pseudomonas aeruginosa strain CLN1_16S ribosomal RNA gene, partial sequence	MN830401.1	100	99.93	0.0	2580

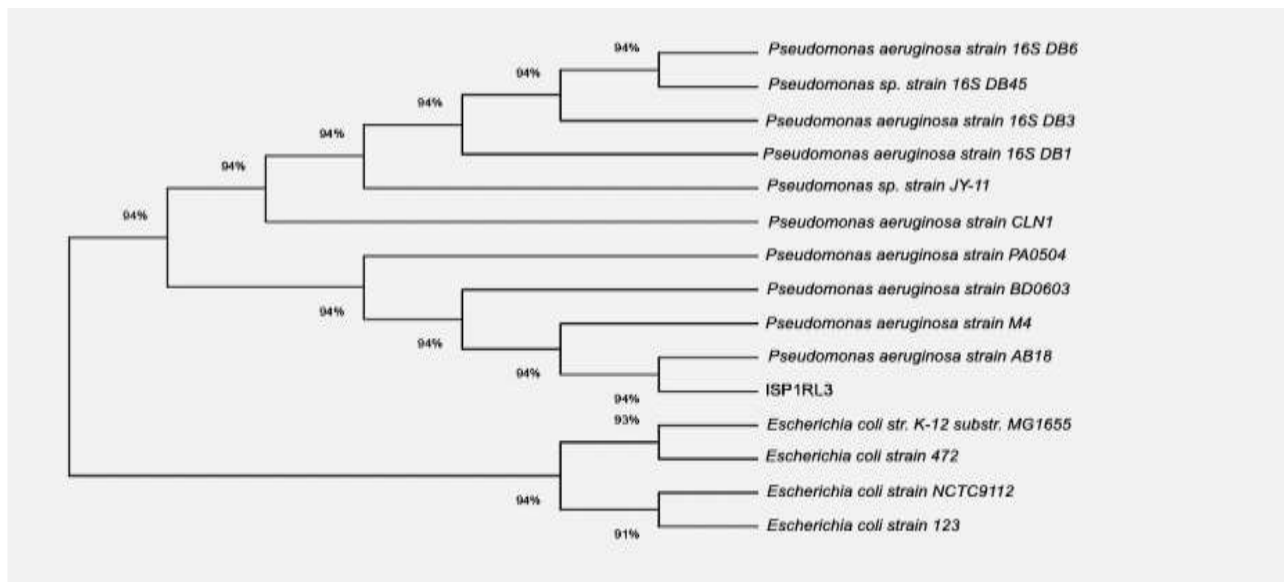


Figure 2. The phylogenetic tree of isolate ISP1RL3 which describes the phylogenetic position of isolate ISPIRL3 with the bacterium *Pseudomonas aeruginosa* strain AB18. Note: The phylogenetic tree construction is based on the Neighbor-joining tree statistical model with a bootstrap value of 1000 repetitions using the Kimura-2 parameter model.

Ethyl acetate extract of P. aeruginosa ISP1RL3 inhibited all non-resistance and MDR bacterial targets. This growth inhibition was indicated by the presence of a clear zone around the paper disc containing the ethyl acetate extract of ISP1RL3 which was placed over the spread of the tested bacterial isolates (Figure 3). The non-resistant bacterial targets were inhibited at range of 12-14 mm (Table 2) and the MDR bacteria was inhibited with an average diameter zone of inhibition of 10 mm (Table 3).

Table 2. Diameter of the inhibition zone of ISP1RL3 bacterial isolate against non-resistant pathogenic bacteria

Sample	Inhibition Zone (mm)			
	S. aureus ATCC 25923	S. mutans FNCC 0405	E. coli ATCC 25922	K. pneumoniae ATCC 700603
Ethyl acetate extract ISP1RL3	13,5±4,3	14,3±3,1	12,2±0,4	13,5±1,7

Table 3. Diameter of the inhibition zone of ISP1RL3 bacterial isolate against multidrug resistant pathogenic bacteria

Sample	Inhibition Zone (mm)			
	MRSA	E. coli ESBL	K. pneumoniae ESBL	Acinetobacter
Ethyl acetate extract ISP1RL3	10,0±0,4	10,8±0,1	10,5±0,2	10,0±0,2

Note: S. aureus: Staphylococcus aureus ATCC 25923; S. mutans: Streptococcus mutans FNCC 0405; E. coli : Escherichia coli ATCC 25922; K. pneumoniae : Klebsiella pneumoniae ATCC 700603; MRSA: Methicillin-resistant Staphylococcus aureus; E. coli ESBL: Escherichia coli ESBL; K. pneumoniae ESBL: Klebsiella pneumoniae ESBL

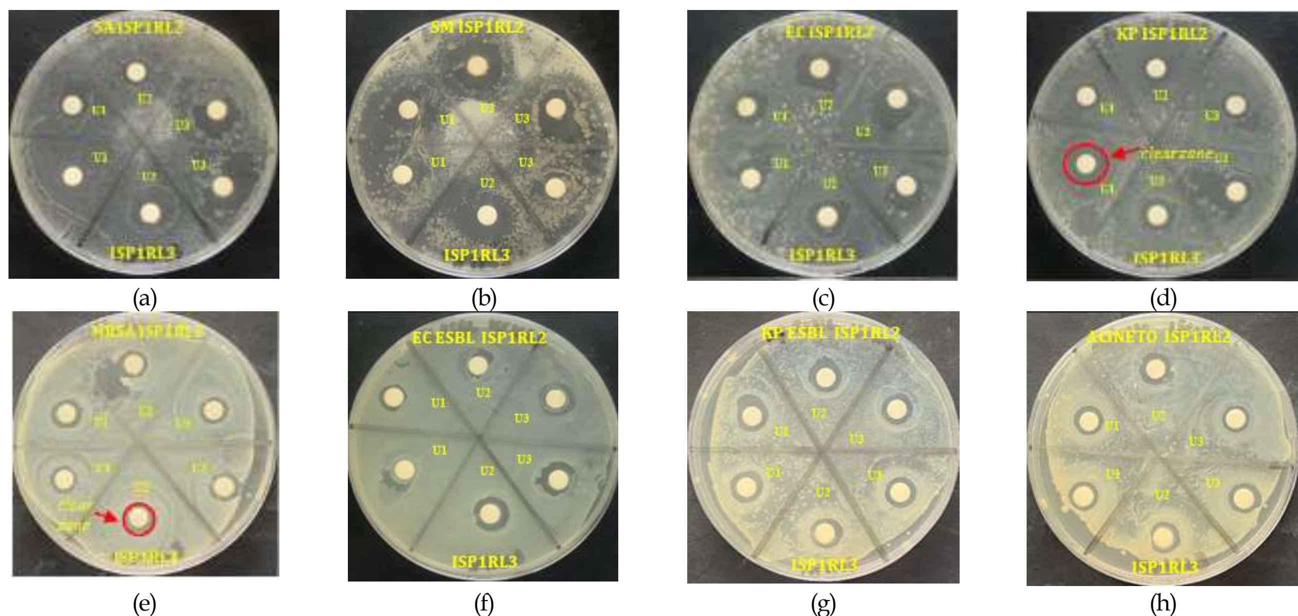


Figure. 3. Antibacterial activity of ethyl acetate extract isolate ISP1RL3 against non-resistant pathogenic bacteria: (a) S. aureus ATCC 25923; (b) S. mutans FNCC 0405; (c) E. coli ATCC 25922; (d) K. pneumoniae ATCC700603 and against multidrug resistant pathogenic bacteria; (e) Methicillin-resistant Staphylococcus aureus; (f) Escherichia coli ESBL; (g) Klebsiella pneumoniae ESB; and (h) Acinetobacter. Note: red arrows : clear zone; U1 : first repetition; U2: second repeat; U3: third repetition.

The separation of ethyl acetate crude extracts ISP1RL3 based on TLC gave five five fractions with varying Rf values of each fraction, as shown in Figure 4. Evaluation of these five fractions could inhibited majority of bacterial test organisms, except for fraction 5 which could not inhibit the growth of K. pneumoniae ATCC 700603 (Table 4 and Table 5). Nevertheless, the result confirmed that indeed the active metabolite synthesized by P. aeruginosa ISP1RL3 has strong antibacterial activity both against non-resistant and MDR bacterial targets.

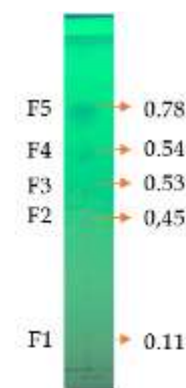


Figure 4. ISP1RL3 thin layer chromatography results. Note: F1-F5: fraction one to five; red arrows: Rf value of each fraction.

Table 4. Diameter of the inhibition zone of ISP1RL3 bacterial isolate against non-resistant pathogenic bacteria

Fraction	Inhibition Zone (mm)			
	S. aureus ATCC 25923	S. mutans FNCC 0405	E. coli ATCC 25922	K. pneumoniae ATCC 700603
1	16.6±0.1	16.9±0.9	17.8±1.1	18.2±0.3
2	11.9±0.6	17.3±0.9	11.1±0.4	14.4±0.0
3	10.6±0.0	19.1±0.3	11.4±0.1	13.8±1.4
4	15.3±2.9	12.2±0.3	11.4±0.0	7.5±0.2
5	11.9±0.6	16.3±0.2	11.7±0.3	0

Table 5. Diameter of the inhibition zone of ISP1RL3 bacterial isolate against multidrug resistant pathogenic bacteria

Fraction	Inhibition Zone (mm)			
	MRSA	E. coli ESBL	K. pneumoniae ESBL	Acinetobacter
1	10.1±0.5	11.8±0.2	11.8±0.1	12.4±0.3
2	12.4±0.5	11.8±0.4	12.5±0.4	11.4±0.2
3	13.7±1.0	12.7±0.5	11.2±0.2	10.3±0.3
4	13.2±0.5	12.7±1.1	11.6±0.1	10.2±0.1
5	10.1±0.1	11.0±0.1	14.7±3.1	14.8±1.5

Note: *S. aureus: Staphylococcus aureus* ATCC 25923; *S. mutans: Streptococcus mutans* FNCC 0405; *E. coli : Escherichia coli* ATCC 25922; *K. pneumoniae : Klebsiella pneumoniae* ATCC 700603; *MRSA*: Methicillin-resistant *Staphylococcus aureus*; *E. coli* ESBL: *Escherichia coli* ESBL; *K. pneumoniae* ESBL: *Klebsiella pneumoniae* ESBL. The average diameter zone of inhibition for each bacterial test was measured from four replications.

Table 6. Antibacterial compounds detected in the ethyl acetate extract of P. aeruginosa ISP1RL3 isolate

Compound name	Compound characteristic	Activity	Peak area (%)	Reference
o-Xylene	Organic compound	Antibacterial	5.27	(Tiwari et al., 2016; Zayed & Samling, 2016)
Ethylbenzene	Organic compound	Antibacterial	6.52	(Bellahcen et al., 2019)
p-Xylene	Organic compound	Antibacterial	9.20	(Morah & Odey, 2020)
Benzene, 1,3-dimethyl-	Organic compound	Antibacterial	9.20	(Wei & Zhang, 2023)
Propanoic acid, 3-ethoxy-, ethyl ester	Organic compound	Antibacterial	0.39	(Haque *et al.*, 2009)
D-Limonene	Organic compound	Antibacterial	0.55	(Han et al., 2020)
Acetic acid, phenylmethyl ester	Organic compound	Antibacterial	0.15	(Garciglia-Mercado et al., 2021)
Citral	Organic compound	Antibacterial	0.09	Shi 2021, qian 2020
Naphthalene	Organic compound	Antibacterial	0.09	(Rokade & Sayyed, 2011)
Estragole	Organic compound	Antibacterial	0.37	(AlBalawi et al., 2023)
Dodecanoic acid	Organic compound	Antibacterial	0.13	(Yoon et al., 2018)
Carvacrol, TMS derivative	Organic compound	Antibacterial	0.14	(Kachur & Suntres, 2020)
Thymol, TBDMS derivative	Organic compound	Antibacterial	0.16	(Kachur & Suntres, 2020)

The GC-MS analysis of the crude ethyl acetate extract showed that there were 109 active compound peaks detected as shown in Figure 4. Among the 109 peaks that were present, 13 active compounds were reported to display antibacterial activity (Table 6).

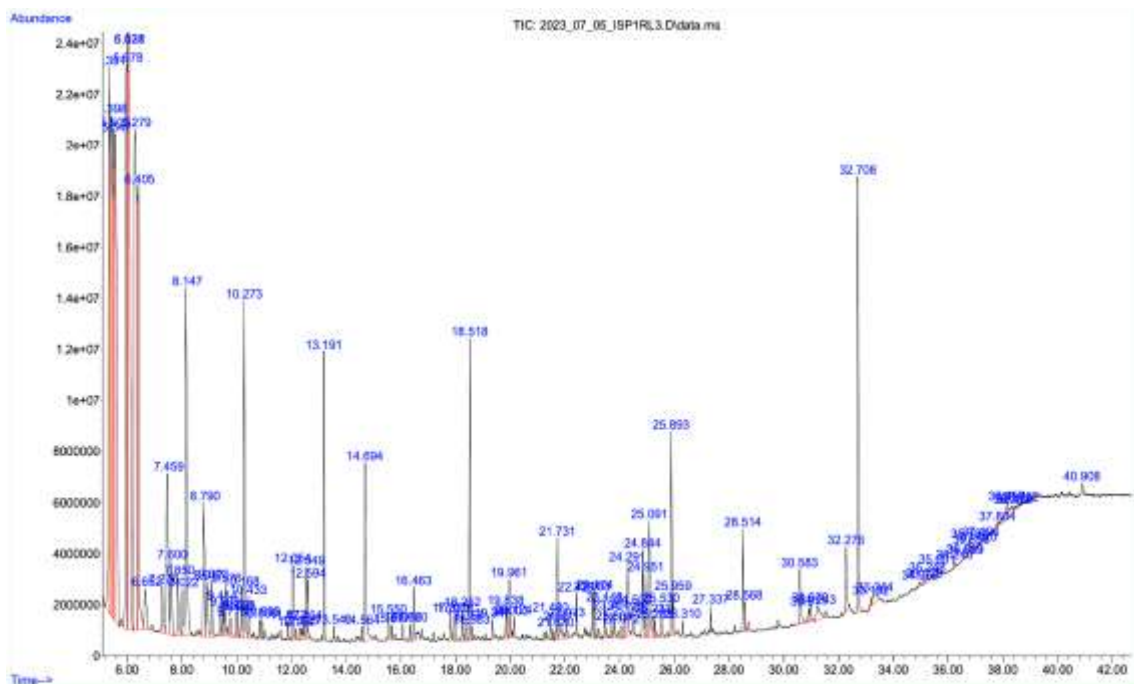


Figure 4. GC-MS chromatogram of the ethyl acetate extract of ISP1LR3 isolate

Discussion

The emergence of multidrug resistance bacteria are of important health issues that urgently need to be overcomed. This present study described antibacterial evaluation of an isolate encoded as ISP1RL3 which was isolated from seaweed Eucheuma cottonii, with potential as antibacterial producer. Morphological features of the isolate observed under light microscope and SEM were rod structure with Gram negative cell wall. This morphological characteristics were confirmed with molecular identification showed identity of the isolate ISP1RL3 as Pseudomonas aeruginosa. The fact that the isolate was purified from seaweeds is not suprising given that Pseudomonas spp, including P. aeruginosa has been reported to occupy a wide range of marine habitats such as marine sediments, seawaters, marine plants and animals (Bollinger et al., 2020; Elabed et al., 2019).

The result obtained in this study has strengthen a preliminary antibacterial screening using block agar method of P. aeruginosa ISP1RL3 as previously reported (Purnami et al., 2022). The ethyl acetate extract of P. aeruginosa ISP1RL3 has consistently inhibited bacterial tests both for non-resistance strains and MDR bacterial strains. The observed antibacterial result showed that the metabolites extracted in the ethyl acetate extract could be grouped as broad spectrum (Thenmozhi et al., 2014). This finding in line with previous study that highlighted a broad bioacitivity of P. aeruginosa against a number of Gram positive and negative bacteria (Amankwah et al., 2022; Lee et al., 2013). With regards to MDR bacterial strains, the ethyl acetate extracts produced a roughly similar zone of inhibition of 10 mm. Although ISP1RL3 was able to inhibit the growth of Gram negative E. coli ESBL with the highest inhibition zone, ISP1RL3 isolate was also able to inhibit the growth of MRSA. This is because Gram-positive bacteria do not have an outer membrane consisting of lipopolysaccharide, which functions as an additional protective layer like Gram- negative bacteria, causing Gram-positive bacteria to be more sensitive to antibacterial compounds (Epand et al., 2016; Ouchari et al., 2019). The difference in the diameter of the inhibition zone is thought to depend on the secondary metabolites produced by the isolate. This assumption is supported by Dharmawan, et al. (2009) who stated that variations in the diameter of the clear zone occured because each bacterium produces different types of secondary metabolites with different chemical structures, compounds, chemical concentrations, and differences in the polarity of the compounds contained in the bacteria, and differences in the morphological and physiological properties of each test bacteria (Dharmawan et al., 2009).

Conversely, the observed antibacterial activity was relatively higher against non-multi drug resistance. Such discrepancy could be likely related to the fact that MDR bacteria have in general more resilience against antibiotics via resistance gene present in their plasmid. However, the obtained results provided the evidence of anti MDR activity posseses by the bacteria. Although in some cases,P. aeruginosa has been associated as microbial pathogen (Qin et al., 2022; Tuon et al., 2022), a number of studies have reported vast arrays of secondary metabolites and antibiotics that are present in

the genome of this bacterial species such polyketide synthase and non-ribosomal peptide synthetase (Alam et al., 2021; Isnansetyo & Kamei, 2009; Kung et al., 2010).

Analyis of GC-MS from the crude ethyl acetate extract of marine Pseudomonas aeruginosa ISP1RL3 revealed a number of secondary metabolites that have been associated with antibacterial activity such as terpenoids (D-limonene, carvacrol, thymol), phenolics (p-Xylene, Benzene, 1,3-dimethyl, Ethylbenzene, o-Xylene), fatty acids (Acetic acid, dodecanoic acid), as summarized in Table 2. Uniquely, although present in small concentrations, citral compounds (0.09%) were found in an ethyl acetate extract from the marine P. aeruginosa bacterium ISP1RL3 which is usually found in plant essential oils. This compound has been reported to inhibit the growth of carbapenem-resistant bacteria Enterobacter cloacae (Qian et al., 2020).

The ethyl acetate extracts also contained naphthalane and dodecanoic acid (lauric acid) which are very potent in inhibiting the growth of various human pathogenic bacteria including multidrug resistant bacteria MRSA (Rokade & Sayyed, 2011; Yoon et al., 2018). Furthermore, the presence of estragole (0.37%) in the ethyl acetate extract was suprising because the compound was also found to be the most abundant compound(66.85%) in the extract of aniseed plants (Pimpinella anisum) which have been reported to display antibactericidal MIC value of 0.170 mg/mL and bacteriostatic MBC value of 0.340 mg/mL against Acinetobacter baumannii respectively (AlBalawi et al., 2023).

The detection of acetic acid in the ethyl acetate crude extracts was also linked with antibacterial activity as suggested by research conducted by Garciglia-Mercado, et al. (2021) on exposure of acetic acid (4%) as a treatment to disinfect Acinetobacter baumanii. Acetic acid as a weak acid can cross the bacterial membrane more easily it will dissociate, acidify the cytoplasm, which can cause acid-induced protein, membrane, and DNA damage and thus create physical changes in the cell wall of A. baumanii (Garciglia-Mercado et al., 2021).

The presence of two active compunds carvacrol (0.14%) and thymol (0.16%) was also associated with antibacterial activity. These two compounds are generally considered safe for consumption and have been shown to be potent antibiotic agents against a wide range of Gram positive and negative bacteria through several mechanisms including disrupting the bacterial membrane, inhibition of efflux pumps (transmembrane proteins), prevention, formation of preformed biofilms, inhibition of bacterial motility, and inhibition of membrane ATPase (Kachur & Suntres, 2020).

Conclusion

The bacterial isolate ISPRL3 which was cultivated from seaweed E. cottonii has been assigned as Pseudomonas aeruginosa and ethyl acetate extracts evaluation has confirmed ability of the isolate to synthesize broad spectrum antibacterial compounds against multi drug resistace and non-resistance bacterial target. This finding provided an importance insight on the high potential of seaweed-associated bacteria as antibacterial producers. Further research should be focused to optimized the growth of P. aeruginosa ISP1RL3 with regards to produce antibacterial compounds. Furthemore, structure elucidation is required to specifically determine the precise antibacterial compounds synthesized by P. aeruginosa ISP1RL3.

Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Conflicts of Interest

The authors declare no conflict of interest.

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